OPTIMIZATION OF CALLOGENESIS BY COMBINATION OF 2,4-D AND KINETIN IN APRICOT TISSUES *(Prunus armeniaca* L.) CULTURED IN AURES, ALGERIA

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Abstract. Apricot orchards are a vital part of Algeria's agricultural economy. However, production levels are inconsistent, and low yields are largely due to aging trees and the general decline of orchards. In this context, callus induction plays a vital role in orchard renewal by enabling the mass propagation of disease-free plants and helping to rejuvenate apricot orchards while preserving local genetic resources. This study aimed to improve the efficiency of callus induction in apricot tissue culture by evaluating the effects of various combinations of plant growth regulators. *Prunus armeniaca* L. leaf explants were cultured on Murashige and Skoog (MS) medium supplemented with various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and kinetin. The results showed that a sterilization protocol using a combination of bleach and potassium permanganate most effectively disinfected both leaf and bud explants. The highest callus induction rates were achieved with MS9 and MS8 media, containing 10 mL of 2,4-D and 3 or 2 mL of kinetin, resulting in callus formation rates of 94.44% and 88%, respectively. Three types of callus were also obtained: friable, compact, and nodular, which varied in color. Callogenesis from leaf explants primarily occurred as cream-colored cell clusters that formed along the veins and excision zones. In conclusion, this study demonstrates that an MS medium supplemented with optimized concentrations of 2,4-D and kinetin effectively promotes callogenesis in *Prunus armeniaca* L. This offers a promising approach for renewing and sustainably propagating apricot orchards in Algeria.

Key words: 2,4-D; apricot; callogenesis; kinetin; morphogenetic; *Prunus armeniaca* L.

INTRODUCTION

Apricots (*Prunus armeniaca*) are highly nutritious fruits valued for their numerous health benefits and economic significance. As functional foods, they contribute to a balanced diet and offer opportunities for sustainable development in agriculture and industry. Notably, ongoing research into the bioactive compounds in apricots is expanding our understanding of their therapeutic potential [20, 46], placing this fruit in a strategic position for several countries. Globally, apricot production remains substantial, with an estimated yield of approximately 3.86 million tonnes in 2022 - reflecting a 6.6% increase from 2021. The top five producing countries - Turkey, Uzbekistan, Iran, Algeria and Italy - account for over 53% of global output, with Algeria ranking fourth globally and first in Africa [37, 48].

The city of N'Gaous in the Batna province of Algeria is notable for its historical and cultural connection to apricot cultivation. Apricots represent a vital economic resource and a regional cultural symbol. However, the productivity and longevity of apricot orchards in this region are under threat from ageing mother plants and progressive aridification of the climate. These factors threaten both yield and genetic diversity [38]. In order to renew and intensify apricot orchards in arid and semi-arid zones such as Batna, it is crucial to enhance propagation methods and encourage the regeneration of superior genotypes.

Plant tissue culture, particularly the induction of callogenesis, is an effective strategy for overcoming plant ageing and ensuring the sustainable propagation of elite apricot cultivars. Callogenesis - the formation of undifferentiated callus tissue from explants - is a critical step in plant biotechnology, forming the basis for regeneration, genetic transformation and secondary metabolite production. This process is influenced by multiple factors, especially the type and concentration of plant growth regulators (PGRs), such as auxins and cytokinins [14].

2,4-Dichlorophenoxyacetic acid (2,4-D) and kinetin are particularly well-known for their ability to promote callus formation. 2,4-D, a synthetic auxin, enhances cell division and callus development. Effective concentrations generally range from 0.5 to 2 mg/L, depending on the species and explant type [24, 42]. Kinetin, a type of auxin, supports cell division and shoot formation, and has been shown to improve callus quality when used alongside 2,4-D. Low concentrations of kinetin (typically 0.1-0.5 mg/L), in combination with optimal levels of 2,4-D, can significantly improve callus induction and biomass accumulation [24, 49]. For example, a mixture of 1.5 mg/L of 2,4-D and 0.2 mg/L of Kinetin has produced notably higher induction rates than either hormone alone [27, 42]. The synergistic action of these plant growth regulators (PGRs) enhances callogenesis and contributes to the production of uniform, vigorous plant tissues, which is critical for large-scale propagation and orchard rejuvenation [12].

The present study therefore aimed to optimise callus induction from apricot explants by evaluating the effects of various concentrations and combinations of 2,4-D and kinetin. This work supports the broader objective of renewing aged apricot orchards through biotechnological means and improving propagation efficiency in the face of increasingly arid conditions expected due to climate change.

MATERIALS AND METHODS

Description of the study area

This study was carried out on *Prunus armeniaca* L., a variety known as Louzi-red, grown in N'gaous region of Algeria. This region is located at the transition between the highlands and the steppe, north-west of the main towns of the Batna district. Its geographical position is (35°32'54.9"N 5°37'08.2"E). It represents a point of transition between the physical and bioclimatic domain, cold semi-arid to the north and east and cool arid to the south. The municipality of N'gaous covers an area of 80.95 km^2, representing 0.66% of the total area of the district.

Harvesting and preparation of explants

Prunus armeniaca L. twigs were harvested in June 2020 from an 8-year-old tree on the Merazgua farm (Batna region). The twigs were stripped of their leaves and the buds were removed. The buds are separated into apical, apex and base buds. Leaves and buds are placed in beakers.

Sterilisation of explants

Two sterilisation protocols were used in order to determine the most suitable protocol with the lowest contamination rate. The first protocol is described as follows: the leaves and buds were rinsed in running water for 20 min and soaked in 70° alcohol for 5 min, then rinsed in sterile distilled water. The plant material is placed in mercuric chloride with the addition of 2 drops of Tween 80, for 15 minutes under vacuum, and then rinsed with sterile distilled water 3 times for 10 minutes each. In the second protocol, briefly, the leaves and buds were rinsed in running water for 20 minutes, then soaked in 70° alcohol for 2 minutes and rinsed with sterile distilled water. The explants were placed in potassium permanganate ($KMnO_4$), 250 mg of which had been dissolved in 1 litre of bleach, to which 3 drops of Tween 80 were added for 15 minutes under vacuum. The plant material was rinsed with sterile distilled water 3 times for 10 minutes each [6].

Composition and search for a culture medium favourable to callogenesis

The basic nutrient medium consists of mineral salts, a carbon source, a gelling agent, and other additives, including growth regulators, vitamins, and amino acids, which are added to enhance the medium's suitability for callogenesis. The basic medium (MS), described by Murashige and Skoog before [29], was utilised. The medium was mixed with stirring and adjusted to pH 5.7 with NaOH (0.1N) or HCl (0.1N), then autoclaved for 20 minutes at 120°C under a pressure of 1 bar. The medium was then dispensed into sterile Petri dishes in a laminar flow hood. In the course of our trials, we tested 14 different combinations. We have used two growth regulators, 2,4-D (2,4-Dichlorophenoxyacetic acid) and Kinetin (6-Furfurylaminopurine) (Table 1). The number of explants used in our experiment was 10 per Petri dish and all experiments were done in triplicate for each treatment.

Culturing

Before each culture, the hood is thoroughly cleaned with 70° alcohol and then disinfected with ultraviolet light for at least two hours. It is important to note that all instruments used for these manipulations are sterilised in a drying oven for one hour at 180°C and then flamed before and after each use to ensure maximum sterility. In the laminar flow hood, the disinfected buds and leaf fragments are carefully dried on sterilised filter paper. The leaves are then carefully cut into small explants and cultured in Petri dishes containing the nutrient medium. Once the explants have been properly cultured, the Petri dishes are sealed with parafilm and kept in complete darkness at a controlled temperature of 28 ± 1°C until callus formation occurs.

Cytohistological analysis of calluses

The cytohistological study is carried out by observation under a light microscope (Carl ZEISS, microscopy GmbH 37081, Germany), and is based on

Table1. Composition of the culture mediums tested in this study for 1000 mL of culture medium

	Macro MS 4.53 g/L (25 mL)	Micro MS 2.25 g/L (5 mL)	Fe-EDTA 0.065 g/L (5 mL)	Vitamins Morel 0.10 g/L (1 mL)	Sucrose 15 g	Glutamine 0.05 mg	Activated charcoal 1.5g	Grow regulators combination (10 mg / 100 mL)
Medium 1	+	+	+	+	+	+	+	2.4-D: 5 mL; Kin.: 0 mL
Medium 2	+	+	+	+	+	+	-	2.4-D: 5 mL; Kin.: 1 mL
Medium 3	+	+	+	+	+	+	-	2.4-D: 5 mL; Kin.: 2 mL
Medium 4	+	+	+	+	+	+	+	2.4-D: 5 mL; Kin.: 3 mL
Medium 5	+	+	+	+	+	+	+	2.4-D: 5 mL; Kin.: 5 mL
Medium 6	+	+	+	+	+	+	-	2.4-D: 10 mL; Kin.: 0 mL
Medium 7	+	+	+	+	+	+	+	2.4-D: 10 mL; Kin.: 1 mL
Medium 8	+	+	+	+	+	+	-	2.4-D: 10 mL; Kin.: 2 mL
Medium 9	+	+	+	+	+	+	+	2.4-D: 10 mL; Kin.: 3 mL
Medium 10	+	+	+	+	+	+	+	2.4-D: 100 mL; Kin.: 0 mL
Medium 11	+	+	+	+	+	+	+	2.4-D: 100 mL; Kin.: 1 mL
Medium 12	+	+	+	+	+	+	+	2.4-D: 100 mL; Kin.: 2 mL
Medium 13	+	+	+	+	+	+	+	2.4-D: 100 mL; Kin.: 3 mL
Medium 14	+	+	+	+	+	+	+	2.4-D: 100 mL; Kin.: 5 mL

Note: +: substace added to the medium; - : substance not added to the medium

the production of sections using a hand-rotated microtome. Inclusion involves embedding the organs in paraffin. Cytohistology of calluses is carried out in several stages: fixation, dehydration, impregnation, inclusion, sections preparation, dewaxing, rehydration, staining and mounting [13].

Statistical analysis

The Shapiro-Wilk (W) test was used to evaluate the normal distribution of the effect of hormonal combinations on callus development, using PAST 4.17 (Statistical analysis software). The significance level was 0.05.

RESULTS

***In vitro* culture technique**

Observation of the cultures of the different explants over time showed contamination of fungal and bacterial origin. Analysis of the following histogram revealed different percentages of contamination depending on the plant explants used (Fig. 1).

The morphogenetic development was clearly visible during callus formation. Under the microscope, the different neoformations appeared as compact callogenic masses of different colours: white, beige and light yellow (Fig. 2). In addition to the colour change, callus proliferation was intense over time (Fig. 3).

Inducing callus formation from leaf explants, or leaf callogenesis, is largely dependent in the kind and quantity of plant growth regulators (PGRs) that are added to the culture medium. Among these regulators, kinetin and 2,4-D, are frequently used to maximize callogenesis and ensuing plant regeneration. The effects of different concentrations and combinations of 2,4-D and kinetin on leaf callogenesis on *Prunus armeniaca* L. were investigated in this study. The figure 2A illustrates callus formation after 15 days and shows also the initiation of whitish callogen masses, while at the same time figure 4A shows the multiplication of callogen masses on leaf veins and leaf excision zones (Fig. 4B).

Of note, this study suggests that the optimal induction of callogenesis and bud formation is achieved by combining 2,4-D and kinetin with certain combinations in MS medium resulting in better callus formation compared to basal media. Indeed, after 8 weeks in culture, callus neoformation was observed in buds (Fig. 5), but only in M9 medium (10 mL 2,4-D; 3

Figure 2. Callus development on M9 culture media (Table 1) as a function of time: after 15 days (**A**), after 1 month (**B**) and after 2 month (**C**), images enlarged by 32X.

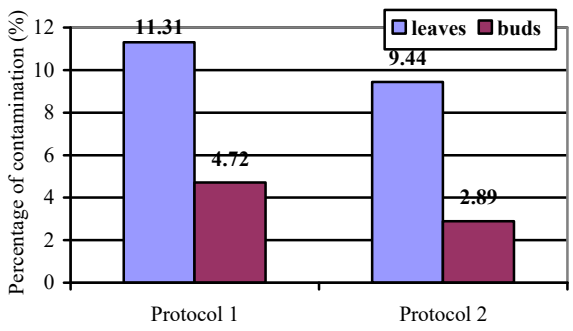

Figure 1. Percentages of contamination of explants (leaves and buds) in culture in relation to the two sterilization protocols tested (Protocol 1: mercuric chloride, Protocol 2: bleach - $KMnO_4$).

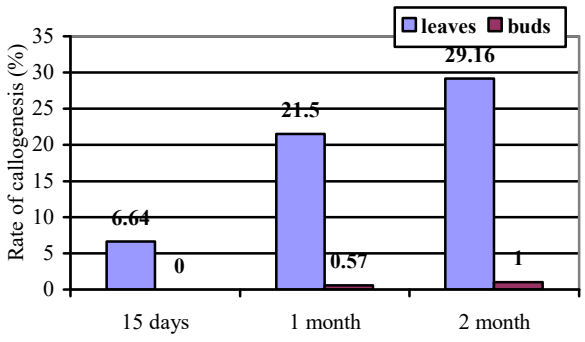

Figure 3. Callogenesis development of leaves and buds as a function of time.

mL kinetin) with an appearance rate of around 1%. The media tested M1 (5 mL 2,4-D; 0 mL kinetin) and M8 (5 mL 2,4-D; 2 mL kinetin) showed earlier callogenesis than the other media. The results indicated that the hormonal combinations in media M1, M2, M3, M4, M5, M8, M9, M10, and M14 facilitated callus induction (Fig. 6). This observation was further corroborated by a statistical analysis, which significantly affirmed the normal distribution of the hormonal effects induced by the combinations utilized in this study (W = 0.803, p<0.01), statistical data are represented in table 2 and figure 7. The presence of 2,4-D alone resulted in the induction of a high percentage of callus, with a rate of 66.66% after four weeks in culture, the callus obtained under these conditions is crumbly, whitish and slightly pasty.

The colour and texture of leaf callus differ according to the growing conditions. By monitoring the development of the callus in the light and in the dark, it was possible to distinguish variations in the intensity of callogenesis and in the state of the callus during its development. The most striking development was noted in the dark) (Fig. 2B compared with the light Fig. 8).

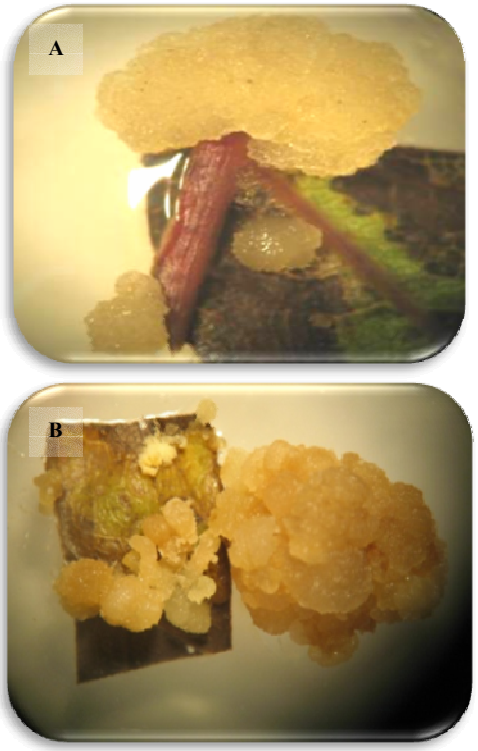

Figure 4. Multiplication of callogenic masses on veins growing on M9 culture media (Table 1) (**A**: enlarged by 32X), and leaf excision zones (**B**: enlarged by 08X).

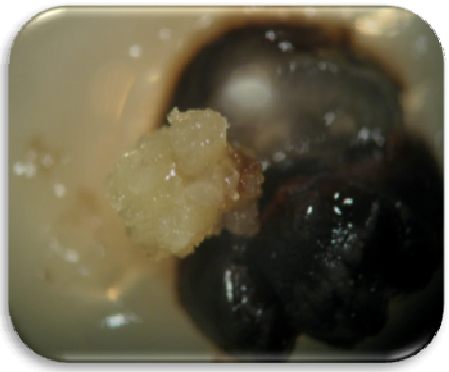

Figure 5. Appearance of callus in buds growing on M9 culture media, image enlarged by 26X.

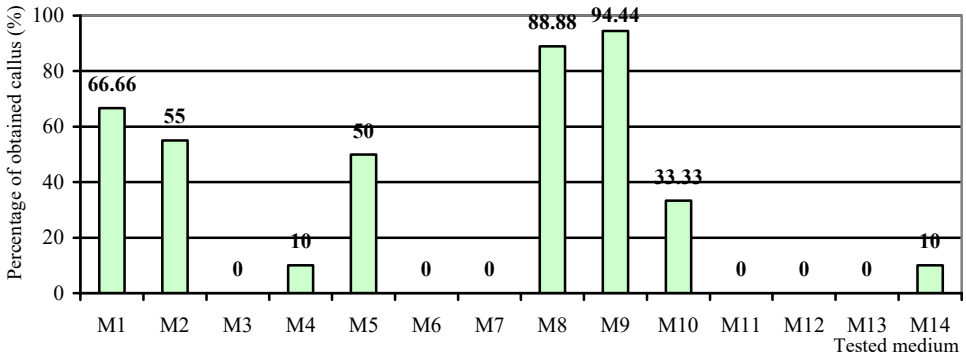

Figure 6. Effects of hormonal combinations on callus development.

Table 2. Descriptive statistics (Data) tested by Shapiro-Wilk (W) to evaluate hormonal effects.

Variable	Observations	Obs. with missing data	Obs. without missing data	Minimum	Maximum	Mean	Standard deviation
Effects of hormonal	14	0	14	0.000	94.440	29.165	35.290

Test of Shapiro-Wilk results: W = 0.803; *p*-value (bilateral) = 0.005; alpha = 0.05
Test interpretation:
H0: The variable from which the sample is drawn follows a Normal distribution;
Ha: The variable from which the sample is drawn does not follow a Normal distribution;
Since the calculated *p*-value is less than the significance level alpha= 0.05, we must reject the null hypothesis H0 and retain the alternative hypothesis Ha;
The risk of rejecting the null hypothesis H0 when it is true is less than 0.54%.

Cytohistological study of callogenesis

Cytohistological study using a light microscope determines the origin and cellular constitution of the calluses tested. Indeed, microscopic observations of callogenic masses obtained during our experiment showed a difference between very intense meristematic zones and others in differentiation (Fig. 9).

DISCUSSION

The primary goal of the current study was to improve the efficiency of callus induction by experimenting with different combinations of two plant growth regulators, kinetin and 2,4-D. The study involved culturing explants on media containing various concentrations of these two hormones. Before achieving the main objective, the explants had to be effectively sterilized. The explants were sterilised using protocols 1 and 2. Both protocols were effective, with contamination rates not exceeding 12%. Protocol 2 was the most effective, with only 3% contamination (Fig. 1). Therefore, protocol 2 was used to sterilise the explants used in callogenesis. A bleach and potassium permanganate combination proved to be the most effective method, which aligns with the findings of Moroccan studies [16] and Boudjemline *et al.* [10]. Mercuric chloride was also effective, as reported by Auge [1] and Bajaj [2]. Potassium permanganate enhances disinfection through its strong oxidative action. Combining it with bleach improves sterilization by targeting reductive contaminants [15]. In this study, after cultuvation of sterilised explants, callus growth was continuous throughout the experiment. It reached its maximum after 60 days of culture for the fourteen types of culture medium (Fig. 2 and 3). Similarly, the work of Bekkaye [4] carried out on thurifer juniper callogenesis became more important after the 10th or 12th week. Also, Nurfatimah *et al.* [32] observed callus initiation, six weeks later, when the perivascular cells of the explant leaves began to divide. In the other hand, the trichome (on the second day), roots (on the second or third day), embryoids (on the sixth to eighth day), and buds (on the tenth to fourteenth day) were the morphological structures that were observed to emerge on the callus surface during the induction of morphogenesis in the *Fagopyrum esculentum* callus [41].

In current study, analysis of figure 2A reveals the formation of small swellings in the veins of leaf explants leading to the neoformation of cell clusters after 15 days. This stage leads to the initiation of callogenesis, so it begins with a simple multiplication of superficial cells into small protuberances [36]. Callus initiation occurs in the excision zones and leaf veins (Fig. 4). The consulted data base reported that in globe artichoke, balanced auxin and cytokinin concen-

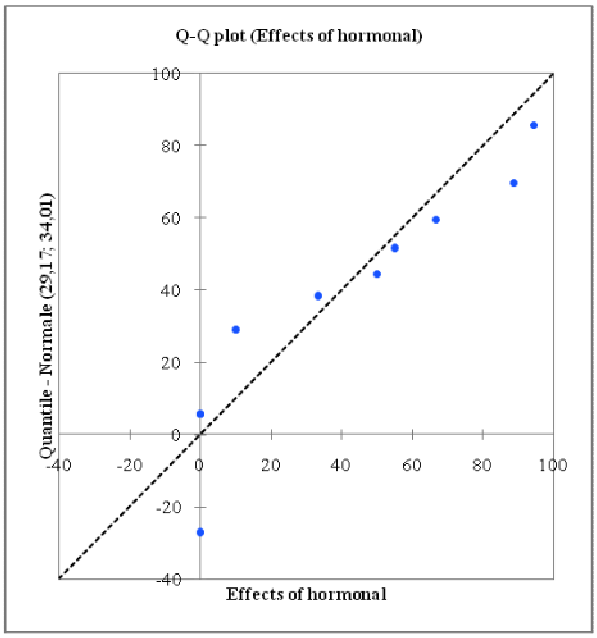

Figure 7. Graphics Q-Q (Normal distribution)

Figure 8. Callus response growing on M9 culture media, after transplanting in the light (image enlarged by 22X).

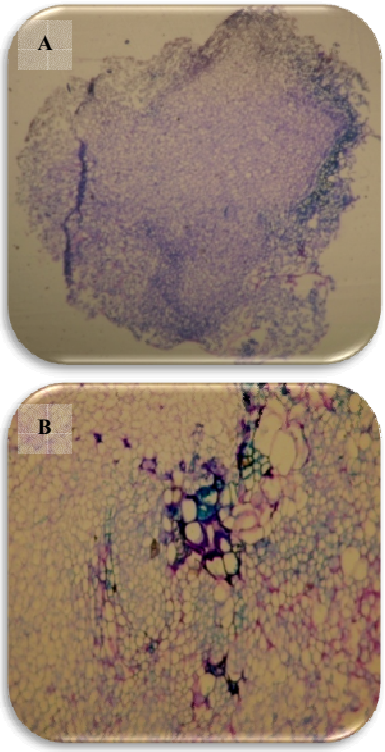

Figure 9. Cytohistological structure (A: image enlarged by 40X) and perivascular origin of callus (B: image enlarged by 100X).

trations (10:1 ratio) were essential for stimulating callogenesis, with *in vivo* leaf explants showing better callus formation and weight [33]. A combination of 2.0 mg/L 2,4-D and 0.2 mg/L kinetin effectively induces callogenesis and somatic embryogenesis in MARDI Siraj 297 rice cultivar, providing an efficient method for genetic manipulation [45].

The success of *in vitro* culture of plant species is influenced by several factors, including the culture medium and the presence of exogenous phytohormones [30]. The results of this work showed that neoformation was observed in buds (Fig. 5) and micropropagation of *Prunus armeniaca* L. when Murashige and Skoog (MS62) medium was used. Generally, all the combinations of growth regulators tested resulted in a variable rate of callogenesis (Fig. 6) and statistically hormonal effects induced by different combinations followed normal distribution (Table 1, Fig. 7). Combinations of growth regulators of the type of mediums: M1, M3, M5, M8, M9 and M10, showed the appearance of callus after 1 month of culture. For mediums M2, M4 and M14, callus appeared after 2 months of culture, which underlines the effectiveness of all the growth regulator combinations tested under our experimental conditions. However, the highest rate of callogenesis was observed in media M1 (5 mL 2,4-D; 0 mL kinetin), M8 (10 mL 2,4-D; 2 mL kinetin) and M9 (10 mL 2,4-D; 3 mL kinetin) with the following percentages: 66.66%, 88.88% and 94.44% respectively. In kodo millet plant, both 2,4-D and picloram in combination with kinetin were effective for callus initiation and somatic embryogenesis, with lower levels of picloram and kinetin promoting organogenic callus with shoot buds [25]. The scientific literature reported that the frequency of bud formation in alfalfa callus is directly proportional to the 2,4-D concentration in the initial medium, with kinetin significantly increasing bud formation [43]. Analysis of figure 7 reveals a variation in the rate of callus formation according to the different growth regulator combinations tested. Fourteen concentrations were used with hormone combinations containing 2,4-D and kinetin. For example, using 5 µM 2,4-D with 10 µM kinetin resulted in a high frequency of callus induction (90.5%) from cotyledon explants [33], according to these last authors the optimal ratio of this combination is 1:5 or 1:10 (kinetin to 2,4-D) have been reported to enhance callus proliferation significantly. The combination of kinetin and 2,4-D is effective for inducing callogenesis in apricot explants when optimized correctly.

The results of this study showed that the combination of 2,4-D and Kinetin that considerably promotes callus formation. Callogenesis initiation trials showed that 2,4-D used at a certain concentration was able to induce callus formation from leaf and bud explants. Our results are similar to those obtained by Tabasum *et al.* [46] using the same phytohormone (2,4-D) on *Zanthoxylum zanthoxyloides* stem and/or leaf fragments to produce callus. In fact, study results reported by Ferradous *et al.* [19] on thurifer showed that the addition of 2,4-D to the culture medium, in combination with Kinetin, gave the best callus induction rate. Similarly, the effectiveness of different hormone combinations can vary significantly based on the species and type of explants used. For instance, the combination of 1 mg/L 2,4-D and 0.5 mg/L Kinetin was noted to induce a higher percentage of callogenesis compared to using either hormone alone [21]**.** Previously, other study results have been reported for certain herbaceous plants such as gerbera (Bolus), where 2,4-D and kinetin appear to be the growth regulators with the greatest influence on callus formation [22], indeed, these hormones showed that callogenesis in Gerbera (*Gerbera gamesonii* Bolus) is induced from leaf fragments or flower *Gerbera gamesonii* stalk segments on a medium containing 2,4-D (1 $mg{\cdot}L^{-1}$) and BA (1 $mg{\cdot}L^{-1}$). In this study, the rest combinations M6, M7, M11, M12 and M13 appear to be ineffective for callogenesis, and these media gave no results. From the results obtained, we can deduce that the different hormone combinations and their concentrations do not have the same effect on callogenesis ability [40].

The rate of callogenesis is higher for leaf explants, as evidenced by good callus growth at this level. These are compact, nodular and creamy. The mass of fresh callus material depends on the environment, the type and position of the explants [22]. The use of growth regulators in the combined state proved effective in inducing callogenic neoformation. However, the frequency with which calluses appear varies according to the type of medium and the type of explants as a function of time. These results are confirmed by work carried out on the Citrange troyer lemon tree by Benyahia *et al.* [5], who revealed that high callus induction percentages were recorded in the presence of a medium containing 2,4-D and kinetin. In a study with *Drosera spatulata*, callus formation percentages reached up to 87.5% when using 0.5 to 2 mg/L of 2,4-D in combination with 0.2 mg/L Kinetin [42]. Another investigation reported that higher concentrations of 2,4-D (up to 5 mg/L) were effective for inducing callus from various explants types, indicating a dose-dependent response where increasing hormone levels generally enhanced callogenesis [24]. In contrast, the work of Pitekelabou *et al.* [35], working on the African peach tree *Nauclea latifolia* claims that after four weeks of cultivation, only leaf fragments grown on MS62 medium containing rooting hormone AIB at 19.69 µM formed white and compact calluses, however, with 2,4-D, no fragments initiated callus. The intensity of callogenesis in explants varies according to the growth regulator composition of the culture media, with media containing 2,4-D giving more callus than media containing naphthalene acetic acid (NAA). These findings are confirmed by several authors, such as Bekkaye [4], who obtained a callus percentage of 3% in a medium containing 3 mg NAA + 2 mg Kinetin [11], reporting that callogenesis is highly dependent on

the nature of the explants, the position of culture and the growth regulator composition of the culture medium. Similarly, Saidi *et al.* [41] and Blidar et *al*. [7], confirmed that the ability of callogenesis differs not only according to the culture medium used, but also according to the culture conditions. In addition, callus colour varies from cream in the dark (Fig. 2B) to dark brown in the light (Fig. 8). These results are confirmed by the work of Saidi *et al.* [41], where the authors showed that leaf explants protected from light produced callus of a whitish beige colour.

The observations highlighted that light can stimulate callus proliferation and growth, and could also contribute to callus formation. In general, early growth requires low light intensity with a 12 to 16 hour photoperiod. For dark-grown tissue, photosynthesis is not a necessary activity, since energy is supplied in the form of carbohydrates [17, 47]. The calluses exposed to light change in appearance, so they have a dark brown colour with a nodular and crumbly texture. This type of callus is morphologically characteristic of non-embryogenic calluses which are capable of undergoing well-defined organogenesis. According to the work of Lutz [26] Nozeran [31] and Blidar et *al*. [8], the initiation of callogenesis seems to be due to the upheaval in the behaviour of the explants, and is essentially linked to the severing of correlations between the explants and the mother plant, as well as to its new environment. Furthermore, according to the work of Margara [28], stem fragments can show abundant callogenesis in continuous darkness.

Indeed, microscopic analysis of figure 9A showed the presence of meristematic cells in clusters around the cribrovascular bundles. These cells are very small with thin walls and dense cytoplasm. The calluses obtained are heterogeneous in appearance, with clusters of small living meristematic cells with large nuclei, a high nucleoplasmic ratio and dense cellular organelles. At the periphery of these clusters are cells differentiated into cambium, underlining the individualisation of these structures in addition to the dispersed differentiated cells (Fig. 9B). The work of Bakhta and Benazzi [3] showed similarly that the origin of these calluses could be perivascular**.** Many other authors have found, in date palms, calluses arise around the vein vessels after the culture of leaf explants under septic conditions that highlighting the existence of initiator cells, named pre-embryogenic indeterminate cells, that are already differentiated but lack embryogenic capacity [18, 34, 44]. In fact, embryogenic cells appear late in the callus produced by the mitotic reactivation of differentiated cells and/or the proliferation of cambiums obtained from root, stem or leaf explants [9, 23, 39].

This study presents a comprehensive approach to optimising callogenesis in *Prunus armeniaca* L. through the combined application of 2,4-D and kinetin, demonstrating both methodological precision and biological relevance. The novelty of the work lies in identifying specific hormone combinations, particularly media M8 and M9 (10 mL 2,4-D with 2-3 mL kinetin), that significantly boost callus induction rates, reaching up to 94.44%. Moreover, the study establishes an effective sterilization protocol using a bleach and potassium permanganate combination, reducing contamination to below 3%. Microscopic analysis revealed early proliferation of superficial cells forming protuberances, with meristematic activity localised near vascular bundles, supporting a perivascular origin of callus formation. These findings underscore the critical roles of explant type and hormonal balance in *in vitro* culture and provide a reproducible protocol for efficient callus production, with promising implications for apricot micropropagation and genetic transformation.

Conflict of interest. There is no actual or potential conflict of interest in relation to this article.

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Received: January 7, 2025
Accepted: June 26, 2025
Published Online: June 29, 2025
Analele Universităţii din Oradea, Fascicula Biologie
https://www.bioresearch.ro/revistaen.html
Print-ISSN: 1224-5119
e-ISSN: 1844-7589
CD-ISSN: 1842-6433
University of Oradea Publishing House